भारतीय मानक

Indian Standard

IS 10972 : 2023

एस्चेरीचिआ कोलाई का डायग्नोस्टिक सेरा तैयार करने की संहिता

(पहला पुनरीक्षण)

Code for Preparation of *Escherichia coli* Diagnostic Sera

(First Revision)

ICS 70.100.99

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Food Microbiology Sectional Committee, FAD 31

FOREWORD

This Indian Standard (First Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Food Microbiology Sectional Committee had been approved by the Food and Agriculture Division Council.

Three types of antigens, that is, Somatic (O-antigens), Capsular (K-antigens) and Flagellar (H-antigens) are known to be present in *Escherichia coli*. So, for complete serotyping, identification of all the antigenic components is necessary. About 188 different types of O-antigens, 103 K-antigens and 56 H-antigens are known in *E. coli*. As such 188 O sera, 103 K sera and 56 H sera are required to be raised, absorbed and finally tested for any cross reactions, before attempting to serotype *E. coli* strains. To avoid confusion as a result of cross reactions, it is appropriate to pay close attention to the minute details at all the stages in the process. The sera must be produced *Only in Rabbits* and in no other animal.

In the preparation of this standard, considerable assistance has been derived from the Central Research Institute Kasauli.

This standard was first published in 1984. The first revision of the standard has been brought out to incorporate following important modifications keeping in view the technological advancements in this area along with the editorial changes to align it in the latest style and format of Indian Standard:

a) List of reference strains of *E. coli* with O, K and H antigens have been updated at Annex A, B and C, respectively, as recommended by the International *E. coli* Centre at the Statens Serum Institute, Copenhagen, Denmark;
b) Storage temperature of immunizing suspensions and serum has been updated from + 4 °C to 2 - 8 °C throughout the standard; and
c) The procedure of 'Bleeding Test' has been modified in **5.3** keeping in view the guidelines issued by CPCSEA (Committee for the purpose of control and supervision of experiments on animals).

The composition of the committee responsible for the formulation of this standard is listed in Annex G.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 2022 'Rules for rounding off numerical values (*second revision*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

CODE FOR PREPARATION OF *Escherichia coli* DIAGNOSTIC SERA

(First Revision)

1 SCOPE

This standard specifies the guideline for raising, absorption and testing of various sera to be used for serotyping of various strains of *Escherichia coli*. This is the guidance document and the actual method may vary depending on the manufacturing procedures validated by individual manufacturers.

2 SELECTION OF IMMUNIZING STRAINS

2.1 Strains used for the preparation of O, K and H sera should have been thoroughly tested for their stable antigenic characteristics and should give minimum possible cross reactions. It is, therefore, advisable to use standard recommended strains which have been tested over the years of experimentation.

2.1.1 *Selection of O-Immunizing Strains* — The International *E. coli* Centre established at the Statens Serum Institute, Copenhagen, Denmark has recommended 188 strains for preparation of O-immunizing suspensions as shown in Annex A.

2.1.2 *Selection of K-Immunizing Strains* — The International *E. coli* Centre has recommended 103 strains for the preparation of K-immunizing suspensions as shown in Annex B.

2.1.3 *Selection of H-Immunizing Strains* — The International *E. coli* Centre has recommended 56 strains for the preparation of H-immunizing suspensions as shown in Annex C.

2.2 The standard recommended strains of *Escherichia coli* can be obtained from The International *E. coli* Centre established at the Statens Serum Institute, Copenhagen, Denmark.

3 REAGENTS

3.1 Buffered Formol Saline (BFS)

a) Stock BFS 2.5 percent

Commercial formalin (40 percent)	50 ml
Physiological saline	2 000 ml

Adjust *p*H to 7.6 by addition of Na_2HPO_4

b) Working BFS 0.25 percent

Stock (2.5 percent) BFS	40 ml
Physiological Saline	360 ml

3.2 Mercuric Iodide Stock Solution (SMIS)

Mercuric iodide	1 g
Potassium iodide	4 g
Distilled water	100 ml

3.3 Bridges Solution

Stock 2.5 percent buffered formol saline (BFS)	2.5 ml
Stock mercuric iodide solution (SMIS)	10 ml
Physiological saline	90 ml

3.4 Acriflavine Solution

Make a 1 : 500 solution in distilled water.

3.5 Phenol Saline, 0.4 Percent

Make from a stock (5 percent) solution of pure phenol.

3.6 Reference Homologous Sera

These are prepared by immunizing rabbits with standard immunizing suspensions and are absorbed to make them monospecific. These should have sufficient titre (not less than 1 : 160) for agglutination tests.

3.7 Formalized Broth Culture

To an 18 h to 20 h broth culture, 0.25 percent of formalin (*v/v*) is added.

4 PREPARATION OF IMMUNIZING SUSPENSION

4.1 O Suspension

The O antigens of *E. coli* are heat stable and are not inactivated by heating at 100 °C for 2 h 30 min.

4.1.1 Before attempting to prepare O suspension, one must be sure that the strain is not rough and does not show auto-agglutination. The recommended O antigen strain is subcultured on nutrient agar (*see* Annex D) from a 4 h nutrient broth culture (*see* Annex D) and incubated at 37 °C overnight.

4.1.2 Next morning, 5 smooth colonies are selected, tested for auto-agglutination in physiological saline and 1 : 500 acriflavin solution.

4.1.3 The above 5 colonies are simultaneously subc-ultured in 10 ml of nutrient broth and incubated at 37 °C for 4 h to 6 h.

4.1.4 Inoculate a well dried agar slope and incubate at 37 °C for 18 h.

4.1.5 Harvest the growth with a sterile bent Pasteur pipette in 1 ml to 2 ml of physiological saline and homogenize.

4.1.6 The above suspension is subjected to heating at 100 °C for 2 h and 30 min and then allowed to cool.

4.1.7 The opacity of the above suspension is adjusted with 0.25 percent buffered formol saline so that it contains $2\ 000 \times 10^6$ organisms per ml.

4.1.8 The immunizing suspension should be stored at 2 °C to 8 °C.

4.2 K Suspension

4.2.1 Inoculate a nutrient agar slope from 4 h to 6 h nutrient broth culture as in **4.1.1**.

4.2.2 Run 2 ml of 10 percent mercuric iodide solution on the slope so as to cover the slope. Allow to stand at 25 °C for 2 h to 3 h for bactericidal action. Harvest the suspension in a sterile tube and adjust the opacity to $8\ 000 \times 10^6$ organisms per ml with Bridge's solution.

4.3 H Suspension

Before preparing H suspension, the standard strains should be given 2 to 3 blind passages in motility test medium (*see* Annex E) to increase motility. At the end of the last passage, the culture should be examined by hanging drop method. For this purpose, a 2 h to 3 h old growth in nutrient broth is examined microscopically by placing a drop of this growth on a cover slip which in turn is placed in inverted position in a grooved glass slide. Not less than 80 percent of bacteria should be motile. Inoculate 100 ml of nutrient broth from this culture and incubate at 37 °C for 6 h to 8 h. The growth is then diluted with 0.25 percent buffered formol saline to an opacity of 400×10^6 organisms per ml using suitable turbidity standards.

4.4 Testing of Immunizing Suspensions

a) The immunizing suspension should be tested for roughness as in **4.1.2**;

b) The immunizing suspension should be tested with a reference homologous sera. It must agglutinate the homologous sera to the extent of the stated titre of the serum; and

c) The immunizing suspension should be tested for sterility.

5 IMMUNIZING AND BLEEDING SCHEDULES

5.1 Selection of Rabbits

Healthy rabbits weighing about 1.5 kg to 2 kg are taken.

5.2 Immunization

5.2.1 Healthy rabbits are intravenously injected with the immunizing suspension, as per the schedule shown below:

Day	Suspension in ml
First	0.25
Third	0.5
Sixth	1.0
Ninth	1.5
Twelfth	2.0
Sixteenth	Bleeding

Different immunization schedule may be used as per the standardized/validated procedure by individual manufacturer.

5.2.2 The rabbits should be examined carefully for any side reaction. Should any reactions occur, further immunization may be discontinued.

5.3 Bleeding

Four to five days after the last injection, the animal is test bled and tested for homologous titres by agglutination test (*see* Annex F). If satisfactory, the animal is bled by cardiac puncture.

NOTE — The animal procedures should be undertaken strictly as per the CPCSEA (Committee for the purpose of control and supervision of experiments on animals) guidelines.

5.4 Blood is collected in dry sterile test tubes. The tubes are kept at 37 °C for 2 h and then in a refrigerator (+ 4 °C) overnight. The clot is removed by centrifugation and all the sera from different bleedings pooled together. Sera showing haemolysis must be discarded.

5.5 The sera should be stored at + 4 °C.

6 PRELIMINARY TESTING OF SERA

Both homologous and heterologous antibody titre of the serum may be determined by tube agglutination method (*see* Annex F). This information may serve as a guideline in choosing strains required for absorption.

7 ABSORPTION

7.1 The absorbing strains (as determined in **6**) should be tested for smoothness as in **4.1.2.** For absorption of O, H and K agglutinins, the absorbing suspensions may be prepared as detailed in **4.1**, **4.2**, and **4.3**.

7.2 For absorption of serum for O agglutinins, approximately one 18 h to 20 h old nutrient agar slope (150 × 16 mm) per ml of the serum is taken. In case of absorption of H agglutinins, centrifuged deposit of an 18 h to 20 h formalized 10 ml broth culture tube per ml of antiserum is taken (*see* **3.7**). For K agglutinin absorption, one 18 h to 20 h nutrient agar slope per ml of serum is sufficient. When the number of strains to be absorbed are more than one, the serum is absorbed in one sitting with all the suspensions prepared from these strains. The suspension(s) (*see* **7.1**) and the serum are thoroughly mixed and kept in a water bath at 52 °C for 2 h after which the mixture is centrifuged and the serum is separated.

7.3 The agglutination test against homologous and heterologous (absorbing strains) antigens is again put up. In case the heterologous antibodies still persist, absorption may be repeated again by taking varied amount of bacterial mass depending on the titre of antibody to be absorbed. Further absorption after the second one is not recommended. For satisfactory results the final homologous titre should not be less than 1 : 160 and the heterologous titre less than 20. The serum is then seitz/membrane filtered and preserved by adding 0.4 percent phenol. It is always advisable to absorb small amounts of serum at one time as unabsorbed sera retain their titre for a longer period of time.

8 FINAL TESTING

8.1 The absorbed serum when tested finally should give the following titre.

8.1.1 *O-Serum*

Homologus titre = not less than 1 : 160 but if more, to be suitably diluted to 1 : 160

Poly H antibody titre = less than 20
Poly K antibody titre = less than 20

8.1.2 *H-Serum*

Homologous titre = not less than 1 : 160 but if more, to be suitably diluted to 1 : 160

Poly O antibody titre = less than 20
Poly K antibody titre = less than 20

8.1.3 *K-Serum*

Homologous titre = not less than 1 : 160 but if more, to be suitably diluted to 1 : 160

Poly O antibody titre = less than 20
Poly H antibody titre = less than 20

9 TEST FOR STERILITY

The sera should be suitably tested for sterility.

10 PRESERVATION AND STORAGE

10.1 The serum should be preserved with 0.4 percent phenol and stored at 2 °C to 8 °C. It shall never be frozen.

10.2 It may be distributed in aliquots of 1 ml each.

11 LABELLING

11.1 The serum should be labelled properly indicating clearly:

a) Name of the manufacturer and manufacturing licence number, if any;
b) Name of the serum shall be written as *E. coli* serum indicating clearly O, H or K type number;
c) Quantity, titre of the serum, the preservative used, if any and the temperature of storage;
d) Batch/lot No. and expiry date; and
e) Label should have the words 'FOR LABORATORY USE ONLY'.

12 MAINTENANCE OF RECORDS

The manufacturer should maintain all relevant records pertaining to production and quality control processes.

ANNEX A

(*Clause* 2.1.1)

REFERENCE STRAINS FOR 'O' ANTIGEN IMMUNIZATION

Strain	Antigens
Reference strain O1	O1 : K1 : H7
Reference strain O2	O2 : K1 : H4
Reference strain O3	O3 : K2ab : H2
Reference strain O4	O4 : K3 : H5
Reference strain O5	O5 : K4 : H4
Reference strain O6	O6 : K2a : H1
Reference strain O7	O7 : K1 : H-
Reference strain O8	O8 : K8 : H4
Reference strain O9	O9 : K9 : H12
Reference strain O10	O10 : K5 : H4
Reference strain O11	O11 : K10 : H10
Reference strain O12	O12 : K5 : H-
Reference strain O13	O13 : K11 : H11
Reference strain O14	O14 : K7 : H-
Reference strain O15	O15 : K14 : H4
Reference strain O16	O16:K1:H-
Reference strain O17	O17 : K16 : H18
Reference strain O18ab	O18ab : K- : H14
Reference strain O18ac	O18ac : K5 : H7
Reference strain O19ab	O19ab : K- : H7
Reference strain O20	O20 : K17 : H-
Reference strain O21	O21 : K20 : H-
Reference strain O22	O22 : K13 : H1
Reference strain O23	O23 : K18ab : H15
Reference strain O24	O24 : K+ : H-
Reference strain O25	O25 : K19 : H12
Reference strain O26	O26 : H-
Reference strain O27	O27 : K- : H-
Reference strain O28ab	O28ab : K- : H-
Reference strain O28ac	O28ac : H-
Reference strain O29	O29 : K- : H10
Reference strain O30	O30 : K- : H-
Reference strain O32	O32 : K- : H19
Reference strain O33	O33 : K- : H-
Reference strain O34	O34 : K- : H10
Reference strain O35	O35 : K- : H10
Reference strain O36	O36 : K- : H9
Reference strain O37	O37 : K- : H10
Reference strain O38	O38 : K- : H26
Reference strain O39	O39 : K- : H-

Strain	Antigens
Reference strain O40	O40 : K- : H4
Reference strain O41	O41 : K- : H40
Reference strain O42	O42 : K- : H37
Reference strain O43	O43 : K- : H2
Reference strain O44	O44 : H18
Reference strain O45	O45 : K1 : H10
Reference strain O46	O46 : K- : H16
Reference strain O48	O48 : K- : H-
Reference strain O49	O49 : K+ : H12
Reference strain O50	O50 : K- : H4
Reference strain O51	O51 : K- : H24
Reference strain O52	O52 : K- : H10
Reference strain O53	O53 : K- : H3
Reference strain O54	O54 : K- : H2
Reference strain O55	O55 : H-
Reference strain O56	O56 : K+ : H-
Reference strain O57	O57 : K- : H-
Reference strain O58	O58 : K- : H27
Reference strain O59	O59 : K- : H19
Reference strain O60	O60 : K- : H33
Reference strain O61	O61 : K- : H19
Reference strain O62	O62 : K- : H30
Reference strain O63	O63 : K- : H-
Reference strain O64	O64 : K- : H-
Reference strain O65	O65 : K- : H-
Reference strain O66	O66 : K- : H25
Reference strain O68	O68 : K- : H4
Reference strain O69	O69 : K- : H38
Reference strain O70	O70 : K- : H42
Reference strain O71	O71 : K- : H12
Reference strain O73	O73 : K- : H31
Reference strain O74	O74 : K- : H39
Reference strain O75	O75 : K95 : H5
Reference strain O76	O76 : K- : H8
Reference strain O77	O77 : K96 ; H-
Reference strain O78	O78 : H-
Reference strain O79	O79 : K- : H40
Reference strain O80	O80 : K- : H26
Reference strain O81	O81 : K97 : H-
Reference strain O82	O82 : K- : H-

Strain	Antigens
Reference strain O83	O83 : K- : H31
Reference strain O84	O84 : K- : H21
Reference strain O85	O85 : K- : H1
Reference strain O86	O86 : K- : H-
Reference strain O87	O87 : K- : H12
Reference strain O88	O88 : K- : H25
Reference strain O89	O89 : K- : H16
Reference strain O90	O90 : K- : H-
Reference strain O91	O91 : K- : H-
Reference strain O92	O92 : K- : H33
Reference strain O93	O93 : - : H16
Reference strain O95	O95 : K+ : H33
Reference strain O96	O96 : K- : H19
Reference strain O97	O97 : K- : H-
Reference strain O98	O98 : K- : H8
Reference strain O99	O99 : K- : H33
Reference strain O100	O100 : K- : H2
Reference strain O101	O101 : K- : H33
Reference strain O102	O102 : K- : H40
Reference strain O103	O103 : K+ : H8
Reference strain O104	O104 : K- : H12
Reference strain O105	O105 : K- : H8
Reference strain O106	O106 : K- : H33
Reference strain O107	O107 : K98 : H27
Reference strain O108	O108 : K- : H10
Reference strain O109	O109 : K- : H19
Reference strain O110	O110 : K- : H39
Reference strain O111	O111 : H-
Reference strain O112ab	O112ab : H18
Reference strain O112ac	O112ac : H-
Reference strain O113	O113 : H21
Reference strain O114	O114 : H32
Reference strain O115	O115 : K- : H18
Reference strain O116	O116:K+ : H10
Reference strain O117	O117 : K98 : H4
Reference strain O118	O118 : K- : H-
Reference strain O119	O119 : H27
Reference strain O120	O120 : K18a : H6
Reference strain O121	O121 : K- : H10
Reference strain O123	O123 : K- : H16
Reference strain O124	O124 : H30
Reference strain O125ab	O125ab : H19
Reference strain O125ac	O125ac : H6
Reference strain O126	O126 : H2
Reference strain O127	O127a : H-

Strain	Antigens
Reference strain O128ab	O128ab : H2
Reference strain O128ac	O128ac : K- : H12
Reference strain O129	O129 : K- : H11
Reference strain O130	O130 : K- : H9
Reference strain O131	O131 : K- : H26
Reference strain O132	O132 : K+ : H28
Reference strain O133	O133 : K- : H29
Reference strain O134	O134 : K- : H35
Reference strain O135	O135 : K- : H-
Reference strain O136	O136 : H-
Reference strain O137	O137 : H41
Reference strain O138	O138 : H14
Reference strain O139	O139 : K12 : H1
Reference strain O140	O140 : K- : H43
Reference strain O141	O141 : K- H4;F4ab
Reference strain O141ac	O141ac : H4
Reference strain O142	O142 : H6
Reference strain O143	O143 : K- : H-
Reference strain O144	O144 : K- : H-
Reference strain O145	O145 : K- : H-
Reference strain O146	O146 : K- : H21
Reference strain O147	O147 : H19;F4ac
Reference strain O148	O148 : K- : H28
Reference strain O149	O149 : H10
Reference strain O150	O150 : K93 : H6
Reference strain O151	O151 : K- : H10
Reference strain O152	O152 : K- : H-
Reference strain O153	O153 : K- : H7
Reference strain O154	O154 : K94 : H4
Reference strain O155	O155 : K- : H9
Reference strain O156	O156 : K- : H47
Reference strain O157	O157 : H19;F4ac
Reference strain O158	O158 : K- : H23
Reference strain O159	O159 : K- : H20
Reference strain O160	O160 : K- : H34
Reference strain O161	O161 : K- : H54
Reference strain O162	O162 : K- : H10
Reference strain O163	O163 : K- : H19
Reference strain O164	O164 : K- : H-
Reference strain O165	O165 : K- : H-
Reference strain O166	O166 : K- : H4
Reference strain O167	O167 : K- : H5
Reference strain O168	O168 : K- : H16
Reference strain O169	O169 : K- : H8
Reference strain O170	O170 : K- : H1

Strain	Antigens
Reference strain O171	O171 : K- : H2
Reference strain O172	O172 : K- : H-
Reference strain O173	O173 : K- : H-
Reference strain O174	O174 : K- : H27
Reference strain O175	O175 : K- : H28
Reference strain O176	O176 : H-
Reference strain O177	O177 : H25
Reference strain O178	O178 : H7
Reference strain O179	O179 : H8

Strain	Antigens
Reference strain O180	O180 : H-
Reference strain O181	O181 : H49
Reference strain O182	O182 : K48 : H25
Reference strain O183	O183 : H18
Reference strain O184	O184 : K- : H11
Reference strain O185	O185 : H28
Reference strain O186	O186 : K- : H-
Reference strain O187	O187 : K- : H52
Reference strain O188	O188 : H10

ANNEX B

(*Clause* 2.1.2)

REFERENCE STRAINS FOR 'K' ANTIGEN IMMUNIZATION

Strain	Antigens
Reference strain K1	O2 : K1 : H4
Reference strain K2ab	O3 : K2ab : H2
Reference strain K3	O4 : K3 : H5
Reference strain K4	O5 : K4 : H4
Reference strain K5	O10 : K5 : H4
Reference strain K6	O4 : K6 : H5
Reference strain K7	O7 : K7 : H4
Reference strain K8	O8 : K8 : H4
Reference strain K9	O9 : K9 : H12
Reference strain K10	O11 : K10 : H10
Reference strain K11	O13 : K11 : H11
Reference strain K12	O4 : K12 : H-
Reference strain K13	O6 : K13 : H1
Reference strain K14	O15 : K14 : H4
Reference strain K16	O17 : K16 : H18
Reference strain K17	O20 : K17 : H-
Reference strain K18a	O23 : K18a : H15
Reference strain K18ab	O23 : K18ab : H15
Reference strain K19	O25 : K19 : H12
Reference strain K20	O21 : K20 : H-
Reference strain K23	O25 : K23 : H1
Reference strain K24	O83 : K24 : H31
Reference strain K26	O9 : K26 : H-
Reference strain K27	O8 : K27 : H-
Reference strain K28	O9 : K28 : H-
Reference strain K29	O9 : K29 : H-

Strain	Antigens
Reference strain K30	O9 : K30 : H12
Reference strain K31	O9 : K31 : H-
Reference strain K32	O9 : K32 : H19
Reference strain K33	O9 : K33 : H-
Reference strain K34	O9 : K34 : H-
Reference strain K35	O9 : K35 : H-
Reference strain K36	O9 : K36 : H19
Reference strain K37	O9 : K37 : H-
Reference strain K38	O9 : K38 : H-
Reference strain K39	O9 : K39 : H9
Reference strain K40	O8 : K40 : H9
Reference strain K41	O8 : K41 : H11
Reference strain K42	O8 : K42 : H-
Reference strain K43	08 : K43 : H11
Reference strain K44	O8 : K44 : H-
Reference strain K45	O8 : K45 : H9
Reference strain K46	O8 : K46 : K30
Reference strain K47	O8 : K47 : H2
Reference strain K48	O8 : K48 : H9
Reference strain K49	O8 : K49 : H21
Reference strain K50	O8 : K50 : H-
Reference strain K51	O1 : K51 : H-
Reference strain K52	O4 : K52 : H-
Reference strain K53	O6 : K53 : H-
Reference strain K54	O6 : K54 : H10
Reference strain K55	O9 : K55 : H-

Strain	Antigens
Reference strain K57	O9 : K57 : H32
Reference strain K83	O20 : K83 : H26
Reference strain K84	O20 : K84 : H26
Reference strain K87	O8 : K87 : H19
Reference strain K92	O73 : K92 : H34
Reference strain K93	O150 : K93 : H6
Reference strain K94	O154 : K94 : H4
Reference strain K95	O75 : K95 : H5

Strain	Antigens
Reference strain K96	O77 : K96 : H-
Reference strain K97	O81 : K97 : H-
Reference strain K98	O107 : K98 : H27
Reference strain K100	O75 : K100 : H5
Reference strain K101	O20 : K101 : H- : F6
Reference strain K102	O8 : K102 : H-
Reference strain K103	O101 : K103 : H-

ANNEX C

(*Clause* 2.1.3)

REFERENCE STRAINS FOR 'H' ANTIGEN IMMUNIZATION

Strain	Antigens
Reference strain H1	O2 : K2ab : H1
Reference strain H2	O43 : K- : H2
Reference strain H3	O53 : K- : H3
Reference strain H4	O2 : K1 : H4
Reference strain H5	O4 : K3 : H5
Reference strain H6	O2 : K1 : H6
Reference strain H7	O1 : K1 : H7
Reference strain H8	O2 : K- : H8
Reference strain H10	O11 : K10 : H10
Reference strain H11	O13 : K11 : H11
Reference strain H12	O9 : K9 : H12
Reference strain H14	O18ab : K- : H14
Reference strain H15	O23 : K18ab : H15
Reference strain H17	O15 : K97 : H17
Reference strain H18	O17 : K16 : H18
Reference strain H19	O9 : K36 : H19
Reference strain H20	O8 : K49 : H20
Reference strain H21	O8 : K49 : H21
Reference strain H23	O45 : Kne : H23
Reference strain H24	O51 : K12 : H24
Reference strain H25	O15 : K16 : H25
Reference strain H26	O131 : K- : H26
Reference strain H27	O15 : Kne : H27
Reference strain H28	O132 : K+ : H28
Reference strain H29	O133 : K- : H29
Reference strain H30	O38 : Kne : H30

Strain	Antigens
Reference strain H31	O3 : K- : H31
Reference strain H32	O114 : H32
Reference strain H33	O11 : Kne : H33
Reference strain H34	O86 : H34
Reference strain H35	O134 : K- : H35
Reference strain H36	O86 : H36
Reference strain H37	O42 : K- : H37
Reference strain H38	O69 : K- : H38
Reference strain H39	O74 : K- : H39
Reference strain H40	O79 : K- : H40
Reference strain H41	O137 : H41
Reference strain H42	O70 : K- : H42
Reference strain H43	O140 : K- : H43
Reference strain H44	O3 : K19 : H44
Reference strain H45	O52 : Kne : H45
Reference strain H46	O26 : H46
Reference strain H47	O86 : Kne : H47
Reference strain H48	O16 : Kne : H48
Reference strain H49	O6 : K13 : H49
Reference strain H51	O8 : K50 : H51
Reference strain H52	O11 : Kne : H52
Reference strain H53	O148a : Kne : H53
Reference strain H54	O161 : K- : H54
Reference strain H55	O75 : Kne : H55
Reference strain H56	O139 : K- : H56

ANNEX D

(*Clause* 4.1.1)

NUTRIENT AGAR

D-1 COMPOSITION

Ingredients	Gms/Litre
Peptone	5.0
Sodium chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5
Agar	15.0

D-2 PREPARATION

Suspend 28.0 g media in 1 000 ml of purified/ distilled water. Adjust final *p*H 7.4 ± 0.2 at 25 °C. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 103 kPa (15 lbs) pressure (121 °C) for 15 min. Cool to 45 °C to 50 °C. Mix well and pour into sterile petri plates.

ANNEX E

(*Clause* 4.3)

MOTILITY TEST MEDIUM

E-1 COMPOSITION

Ingredients	Gms/Litre
Tryptose	10.0
Sodium Chloride	5.0
Agar	5.0

E-2 PREPARATION

Suspend 20.0 g media in 1 000 ml of purified/distilled water. Adjust final pH 7.2 ± 0.2 at 25 °C. Heat to boiling to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 103 kPa (15 lbs) pressure (121 °C) for 15 min. Allow tubed medium to cool to 45 °C to 50 °C in an upright position.

ANNEX F

(*Clause* 5.3)

IMMUNIZING SUSPENSION

F-1 AGGLUTINATION TESTS

Serial two-fold dilutions of the test serum are made in physiological saline and 0.3 ml of each dilution (from 1 : 10 to 1 : 2 560) is pipetted into different Dreyer's tubes. To each tube, 0.3 ml of standard antigen is then added. A control tube containing 0.3 ml of physiological saline and the standard antigen is also included. All the tubes are placed in a water bath for 4 h at 52 °C and then at room temperature for overnight. The last dilution which shows agglutination gives the titre of the antiserum. The control tube should not show any agglutination.

ANNEX G

(*Foreword*)

COMMITTEE COMPOSITION

Food Microbiology Sectional Committee, FAD 31

Organization	*Representative(s)*
ICAR - Indian Veterinary Research Institute, Izzatnagar, Bareilly	DR KIRAN N. BHILEGAONKAR **(*Chairperson*)**
CSIR - Central Food Technological Research Institute, Mysuru	DR ALOK K. SRIVASTAVA DR ASHA MARTIN (*Alternate*)
CSIR - Institute of Microbial Technology, Chandigarh	DR SURESH KORPOLE DR P. ANIL KUMAR (*Alternate*)
Central Research Institute, Kasauli	DR YASHWANT KUMAR DR SUBHADIP MAHAPATRA (*Alternate*)
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Export Inspection Council of India, New Delhi	SHRI WASI ASGHAR SHRI ANGSHUMAN SAHA (*Alternate*)
HiMedia Laboratories Pvt Ltd, Mumbai	DR RAHUL G. WARKE DR GIRISH B. MAHAJAN (*Alternate*)
ICAR - Central Institute for Fisheries Technology, Kochi	DR SATYEN KUMAR PANDA DR B. MADHUSUDAN RAO (*Alternate*)
ICAR - Indian Veterinary Research Institute, Izzatnagar, Bareilly	DR TRIVENI DUTT DR D. K. SINGH (*Alternate*)
ICMR - National Institute of Nutrition, Hyderabad	DR NAVEEN KUMAR R. (*Alternate*)
Marine Products Export Development Authority, Kochi	DR SREENATH P. G. SHRI V. VINOD (*Alternate*)
Merck Life Sciences Pvt Ltd, Mumbai	SHRIMATI SUJATA SAINDANE SHRI SACHIN MALI (*Alternate*)
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Organization	*Representative(s)*
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Member Secretary
SHRIMATI VARSHA GUPTA
SCIENTIST 'D'/JOINT DIRECTOR
(FOOD AND AGRICULTURE), BIS

Panel responsible for Review of Indian Standards related to Microbiological Media Ingredients, FAD 31 : Panel 3

Organization	*Representative(s)*
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Central Research Institute, Kasauli	DR YASHWANT KUMAR
HiMedia Laboratories Pvt Ltd, Mumbai	DR RAHUL G. WARKE
	DR GIRISH B. MAHAJAN
Merck Life Sciences Pvt Ltd, Mumbai	SHRIMATI SUJATA SAINDANE

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